

CLAIMS

What is claimed is:

1. A computer program product readable by a machine having at least one detection mechanism operatively coupled thereto for detecting and measuring at a plurality of different times during a nucleic acid amplification reaction at least one signal whose intensity is related to a quantity of a nucleic acid sequence being amplified in the reaction, the computer program product embodying a program of instructions executable by the machine to perform the steps of:
 - a) deriving a growth curve from measurements of the signal;
 - b) calculating a derivative of the growth curve;
 - c) identifying a characteristic of the derivative; and
 - d) determining a cycle number associated with the characteristic of the derivative.
2. The computer program product of claim 1, wherein the step of calculating a derivative of the growth curve comprises calculating a second derivative of the growth curve, and wherein the characteristic comprises a positive peak of the second derivative.
3. The computer program product of claim 1, wherein the step of calculating a derivative of the growth curve comprises calculating a second derivative of the growth

curve, and wherein the characteristic comprises a negative peak of the second derivative.

4. The computer program product of claim 1, wherein the
5 step of calculating a derivative of the growth curve
comprises calculating a second derivative of the growth
curve, and wherein the characteristic comprises a zero
crossing of the second derivative.

10 5. The computer program product of claim 1, wherein the
step of calculating a derivative of the growth curve
comprises calculating a first derivative of the growth
curve, and wherein the characteristic comprises a
positive peak of the first derivative.

15 6. The computer program product of claim 1, wherein the
step of calculating a derivative of the growth curve
comprises calculating second derivative values of the
growth curve at a number of different cycles in the
20 reaction to yield a plurality of second derivative data
points, the characteristic comprises a positive peak of
the second derivative, and the step of determining the
cycle number associated with the positive peak
comprises:

- 25 i) fitting a second order curve to the second
derivative data points; and
- ii) calculating the cycle number as the location, in
cycles, of a peak of the second order curve.

- 5 7. A computer program product readable by a machine having
at least one detection mechanism operatively coupled
thereto for detecting and measuring at a plurality of
different times during a nucleic acid amplification
reaction at least one signal whose intensity is related
to a quantity of a nucleic acid sequence being amplified
in the reaction, the computer program product embodying
a program of instructions executable by the machine to
10 perform the steps of:
- a) deriving a growth curve from the measurements of the
signal;
 - b) calculating a derivative of the growth curve;
 - c) identifying a characteristic of the derivative; and
 - 15 d) determining a time value associated with the
characteristic of the derivative.
- 20 8. The computer program product of claim 7, wherein the
step of calculating a derivative of the growth curve
comprises calculating the second derivative of the
growth curve, and wherein the characteristic comprises a
positive peak of the second derivative.
- 25 9. The computer program product of claim 7, wherein the
step of calculating a derivative of the growth curve
comprises calculating the second derivative of the
growth curve, and wherein the characteristic comprises a
negative peak of the second derivative.

10. The computer program product of claim 7, wherein the step of calculating a derivative of the growth curve comprises calculating the second derivative of the growth curve, and wherein the characteristic comprises a zero crossing of the second derivative.
11. The computer program product of claim 7, wherein the step of calculating a derivative of the growth curve comprises calculating the first derivative of the growth curve, and wherein the characteristic comprises a positive peak of the first derivative.
12. The computer program product of claim 7, wherein the step of calculating a derivative of the growth curve comprises calculating second derivative values of the growth curve at a plurality of different measurement times in the reaction to yield a plurality of second derivative data points, the characteristic comprises a positive peak of the second derivative, and the step of determining the time value associated with the positive peak comprises:
- i) fitting a second order curve to the second derivative data points; and
 - ii) calculating the time value as the location of a peak of the second order curve.

13. A computer program product readable by a machine having at least one detection mechanism operatively coupled thereto for detecting and measuring signals indicative of the quantities of a target nucleic acid sequence being amplified in a test sample, containing an unknown starting quantity of the target nucleic acid sequence, and of a calibration nucleic acid sequence being amplified in a plurality of calibration samples, containing respective known starting quantities of the calibration nucleic acid sequence, the computer program product embodying a program of instructions executable by the machine to perform the steps of:

a) determining a respective threshold value for the target nucleic acid sequence in the test sample and for each of the known starting quantities of the calibration nucleic acid sequence in the calibration samples, wherein each threshold value is determined for a nucleic acid sequence in a respective sample by:

- i) deriving a growth curve for the nucleic acid sequence from the measured signals;
- ii) calculating a derivative of the growth curve;
- iii) identifying a characteristic of the derivative; and
- iv) determining the threshold value associated with the characteristic of the derivative;

b) deriving a calibration curve from the threshold values determined for the known starting

quantities of the nucleic acid sequence in the calibration samples; and

- c) determining the starting quantity of the target nucleic acid sequence in the test sample using the calibration curve and the threshold value determined for the target sequence.

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14. The computer program product of claim 13, wherein each of the threshold values comprises a cycle number.

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15. The computer program product of claim 13, wherein each of the threshold values comprises an elapsed time of amplification.

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16. The computer program product of claim 13, wherein the step of calculating a derivative of the growth curve comprises calculating a second derivative of the growth curve, and wherein the characteristic comprises a positive peak of the second derivative.

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17. The computer program product of claim 13, wherein the step of calculating a derivative of the growth curve comprises calculating a second derivative of the growth curve, and wherein the characteristic comprises a negative peak of the second derivative.

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18. The computer program product of claim 13, wherein the
step of calculating a derivative of the growth curve
comprises calculating a second derivative of the growth
curve, and wherein the characteristic comprises a zero
crossing of the second derivative.
19. The computer program product of claim 13, wherein the
step of calculating a derivative of the growth curve
comprises calculating a first derivative of the growth
curve, and wherein the characteristic comprises a
positive peak of the first derivative.
20. The computer program product of claim 13, wherein the
step of calculating a derivative of the growth curve
comprises calculating second derivative values of the
growth curve at a number of different measurement
points in the reaction to yield a plurality of second
derivative data points, the characteristic comprises a
positive peak of the second derivative, and the step of
determining the threshold value associated with the
positive peak comprises:
- i) fitting a second order curve to the second
derivative data points; and
 - ii) calculating the threshold value as the location of
a peak of the second order curve.
21. A computer program product readable by a machine having
at least one detection mechanism for measuring:

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- i) signals indicative of the respective quantities of a target nucleic acid sequence in a test sample and of a first internal control being amplified in a first nucleic acid amplification reaction, wherein the first internal control comprises a second nucleic acid sequence different than the target nucleic acid sequence in the test sample;
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- ii) signals indicative of the respective quantities of a first standard and of a second internal control being amplified in a second nucleic acid amplification reaction, wherein the first standard comprises a first known starting quantity of a calibration nucleic acid sequence different than the second nucleic acid sequence, and wherein the
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- second internal control comprises the second nucleic acid sequence;
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- iii) signals indicative of the respective quantities of a second standard and of a third internal control being amplified in a third nucleic acid amplification reaction, wherein the second standard comprises a second known starting quantity of the calibration nucleic acid sequence, the third internal control comprises the second nucleic acid sequence, and the starting quantity
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- of the second nucleic acid sequence is substantially the same in each of the amplification reactions;

wherein the computer program product embodies a program of instructions executable by the machine to perform the

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steps of:

- a) determining a respective threshold value for each of the standards, each of the internal controls,

and the target nucleic acid sequence in the test sample;

- 5 b) normalizing the threshold value determined for the target nucleic acid sequence in the test sample to the threshold value determined for the first internal control;
- c) normalizing the threshold values determined for the first and second standards to the threshold values determined for the second and third
10 internal controls, respectively;
- d) deriving a calibration curve from the known starting quantities and the normalized threshold values of the first and second standards; and
- e) determining the starting quantity of the target
15 nucleic acid sequence in the test sample using the calibration curve and the normalized threshold value determined for the target sequence.

20 22. The computer program product of claim 21, wherein each of the threshold values comprises a cycle number.

25 23. The computer program product of claim 21, wherein each of the threshold values comprises an elapsed time of amplification.

24. The computer program product of claim 21, wherein a
respective threshold value is determined for a nucleic
acid sequence by:
- i) deriving a growth curve from the measured signals;
 - 5 ii) calculating a derivative of the growth curve;
 - iii) identifying a characteristic of the derivative;
and
 - iv) determining the threshold value associated with
the characteristic of the derivative.
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25. The computer program product of claim 24, wherein the
threshold value comprises a cycle number.
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26. The computer program product of claim 24, wherein the
threshold value comprises an elapsed time of
amplification.
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27. The computer program product of claim 24, wherein the
step of calculating a derivative of the growth curve
comprises calculating a second derivative of the growth
curve, and wherein the characteristic comprises a
positive peak of the second derivative.
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28. The computer program product of claim 24, wherein the
step of calculating a derivative of the growth curve
comprises calculating a second derivative of the growth

curve, and wherein the characteristic comprises a negative peak of the second derivative.

- 5 29. The computer program product of claim 24, wherein the step of calculating a derivative of the growth curve comprises calculating a second derivative of the growth curve, and wherein the characteristic comprises a zero crossing of the second derivative.
- 10 30. The computer program product of claim 24, wherein the step of calculating a derivative of the growth curve comprises calculating a first derivative of the growth curve, and wherein the characteristic comprises a positive peak of the first derivative.
- 15 31. The computer program product of claim 24, wherein the step of calculating a derivative of the growth curve comprises calculating second derivative values of the growth curve at a number of different measurement
- 20 points in the reaction to yield a plurality of second derivative data points, the characteristic comprises a positive peak of the second derivative, and the step of determining the threshold value associated with the positive peak comprises:
- 25 i) fitting a second order curve to the second derivative data points; and
- ii) calculating the threshold value as the location of a peak of the second order curve.

32. The computer program product of claim 21, wherein the step of normalizing the threshold value determined for the target nucleic acid sequence in the test sample to the threshold value determined for the first internal control comprises dividing the threshold value determined for the target nucleic acid sequence by the threshold value determined for the first internal control.
33. The computer program product of claim 21, wherein the step of normalizing the threshold values determined for the first and second standards to the threshold values determined for the second and third internal controls, respectively, comprises dividing the threshold values determined for the first and second standards by the threshold values determined for the second and third internal controls, respectively.
34. A computer program product readable by a machine having at least one detection mechanism operatively coupled thereto for detecting and measuring signals indicative of the respective quantities of a first nucleic acid sequence, a first standard, and a second standard being amplified in a reaction vessel, wherein the first standard comprises a known starting quantity of a second nucleic acid sequence different than the first nucleic acid sequence, and wherein the second standard comprises a known starting quantity of a third nucleic acid sequence different than the first and second

sequences, the computer program product embodying a program of instructions executable by the machine to perform the steps of:

- 5 a) determining a respective threshold value for the first nucleic acid sequence, first standard, and second standard;
- b) deriving a calibration curve from the known starting quantities and from the threshold values determined for the first and second standards; and
- 10 c) determining the starting quantity of the first nucleic acid sequence in the test sample using the calibration curve and the threshold value determined for the first nucleic acid sequence.

15 35. The computer program product of claim 34, wherein each of the threshold values comprises a cycle number.

20 36. The computer program product of claim 34, wherein each of the threshold values comprises an elapsed time of amplification.

37. The computer program product of claim 34, wherein a respective threshold value is determined for each nucleic acid sequence by:

- 25 i) deriving a growth curve from the measurements of the signals;
- ii) calculating a derivative of the growth curve;

iii) identifying a characteristic of the derivative;
and

iv) determining the threshold value associated with
the characteristic of the derivative.

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38. The computer program product of claim 37, wherein the
threshold value comprises a cycle number.

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39. The computer program product of claim 37, wherein the
threshold value comprises an elapsed time of
amplification.

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40. The computer program product of claim 37, wherein the
step of calculating a derivative of the growth curve
comprises calculating a second derivative of the growth
curve, and wherein the characteristic comprises a
positive peak of the second derivative.

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41. The computer program product of claim 37, wherein the
step of calculating a derivative of the growth curve
comprises calculating a second derivative of the growth
curve, and wherein the characteristic comprises a
negative peak of the second derivative.

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42. The computer program product of claim 37, wherein the
step of calculating a derivative of the growth curve
comprises calculating a second derivative of the growth

curve, and wherein the characteristic comprises a zero crossing of the second derivative.

5 43. The computer program product of claim 37, wherein the step of calculating a derivative of the growth curve comprises calculating a first derivative of the growth curve, and wherein the characteristic comprises a positive peak of the first derivative.

10 44. The computer program product of claim 37, wherein the step of calculating a derivative of the growth curve comprises calculating second derivative values of the growth curve at a number of different measurement points in the reaction to yield a plurality of second
15 derivative data points, the characteristic comprises a positive peak of the second derivative, and the step of determining the threshold value associated with the positive peak comprises:

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- i) fitting a second order curve to the second derivative data points; and
 - ii) calculating the threshold value as the location of a peak of the second order curve.

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